

NOTES: 66949800

7.5  $\mu$ g DNA

Ricerca 7610-99-9007

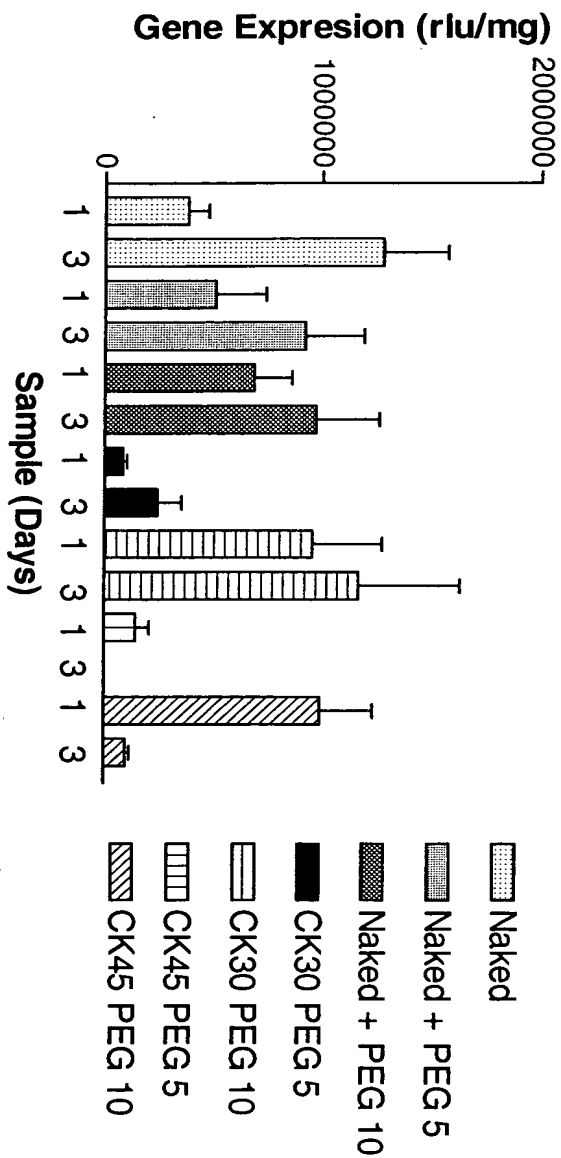


Fig. 1

Pub. No. 6,652,930

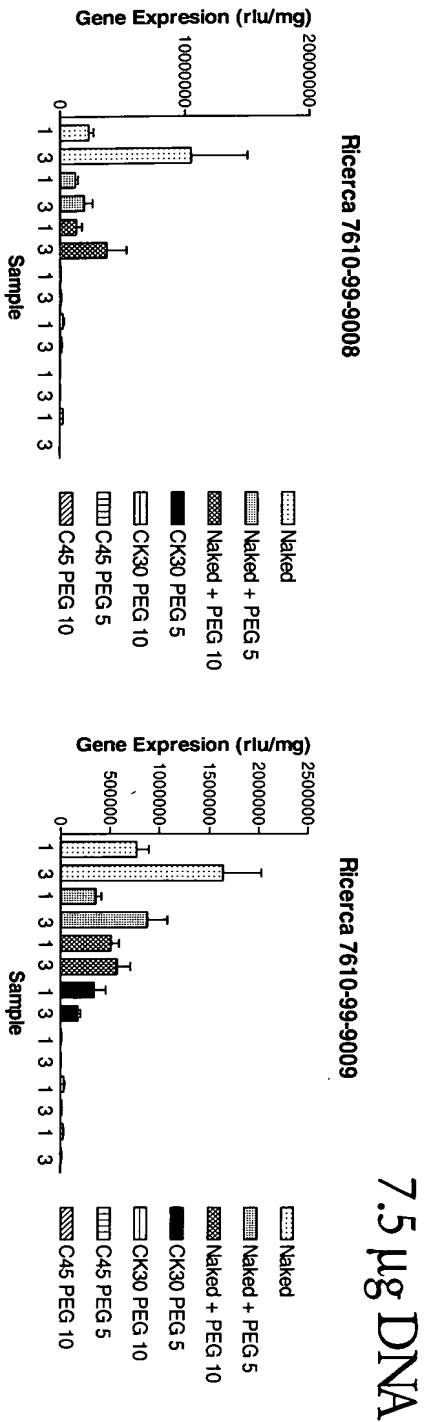


Fig. 2

FIG. 3

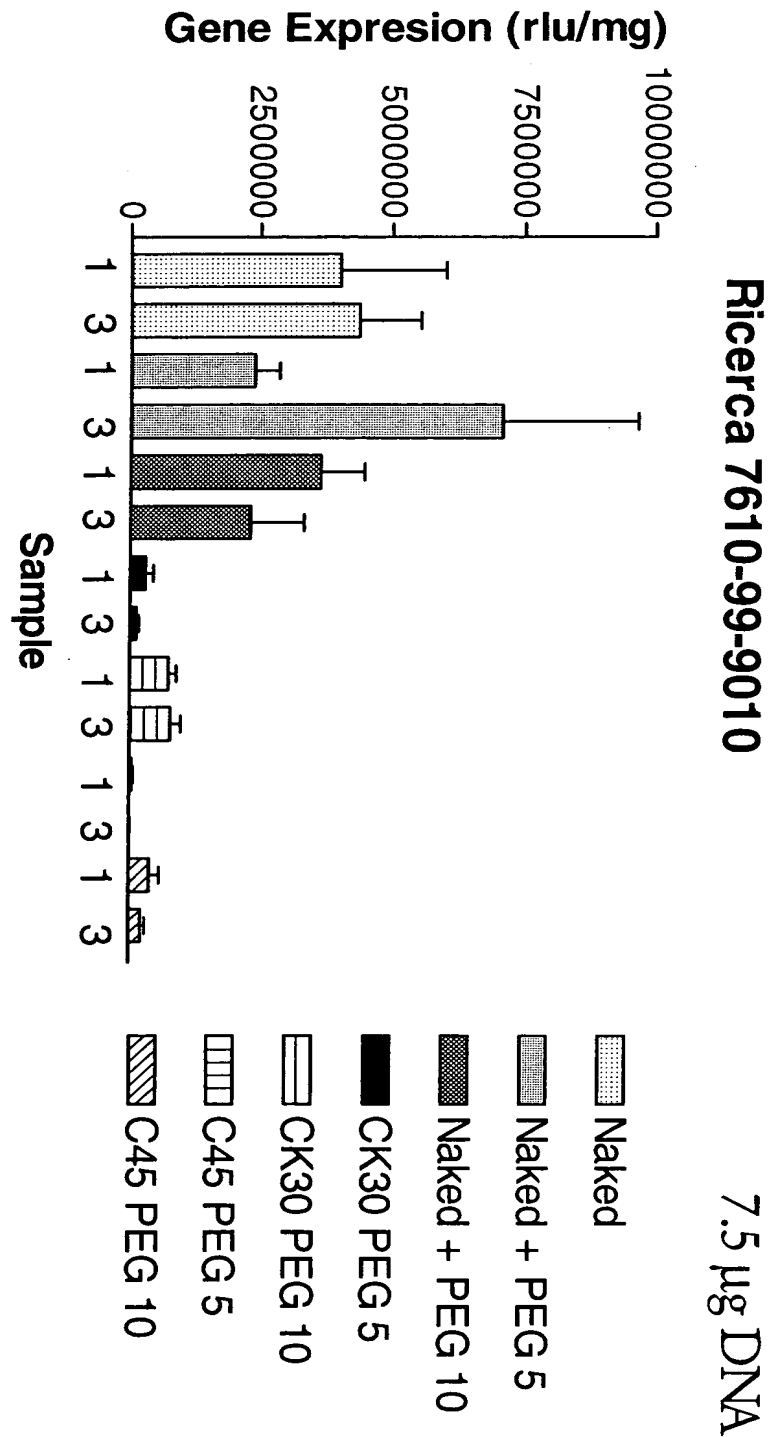


Fig. 3

101650-66923860

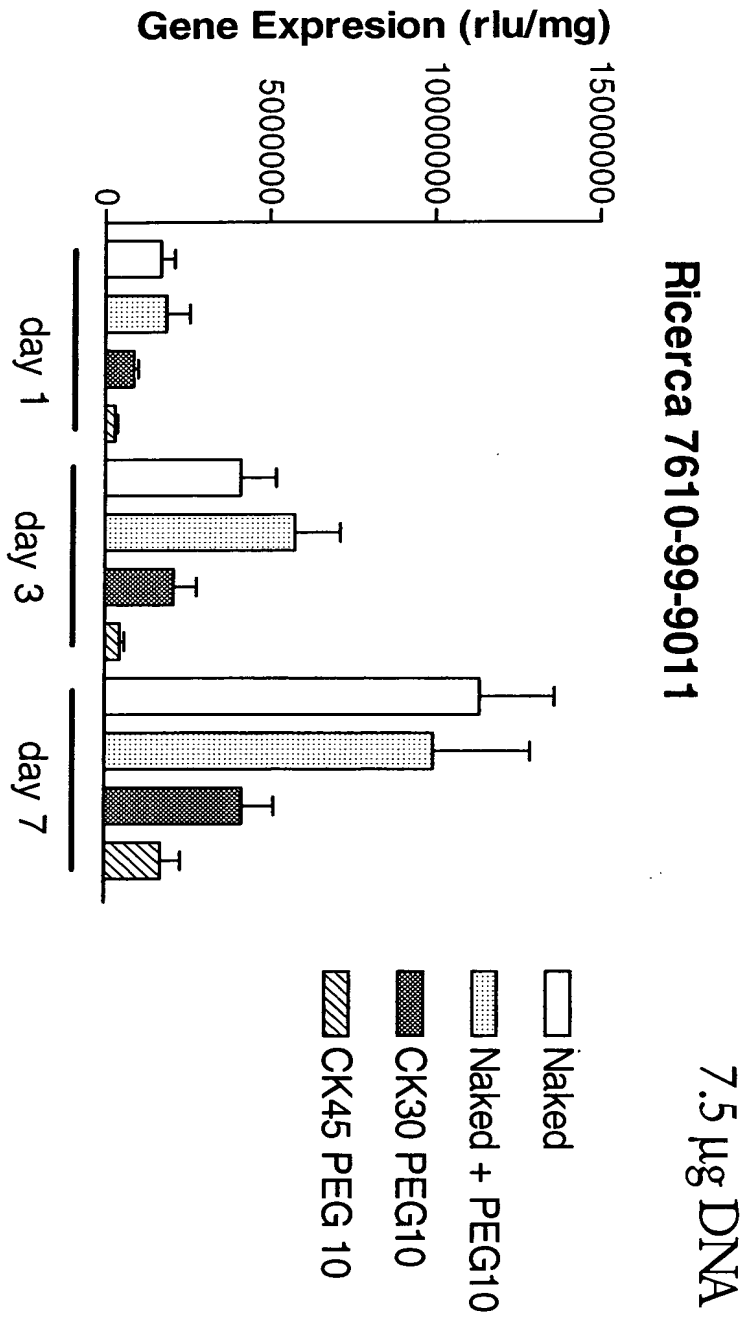


Fig. 4

FILED 65949800

Ricerca 7610-99-9013

7.5  $\mu$ g DNA

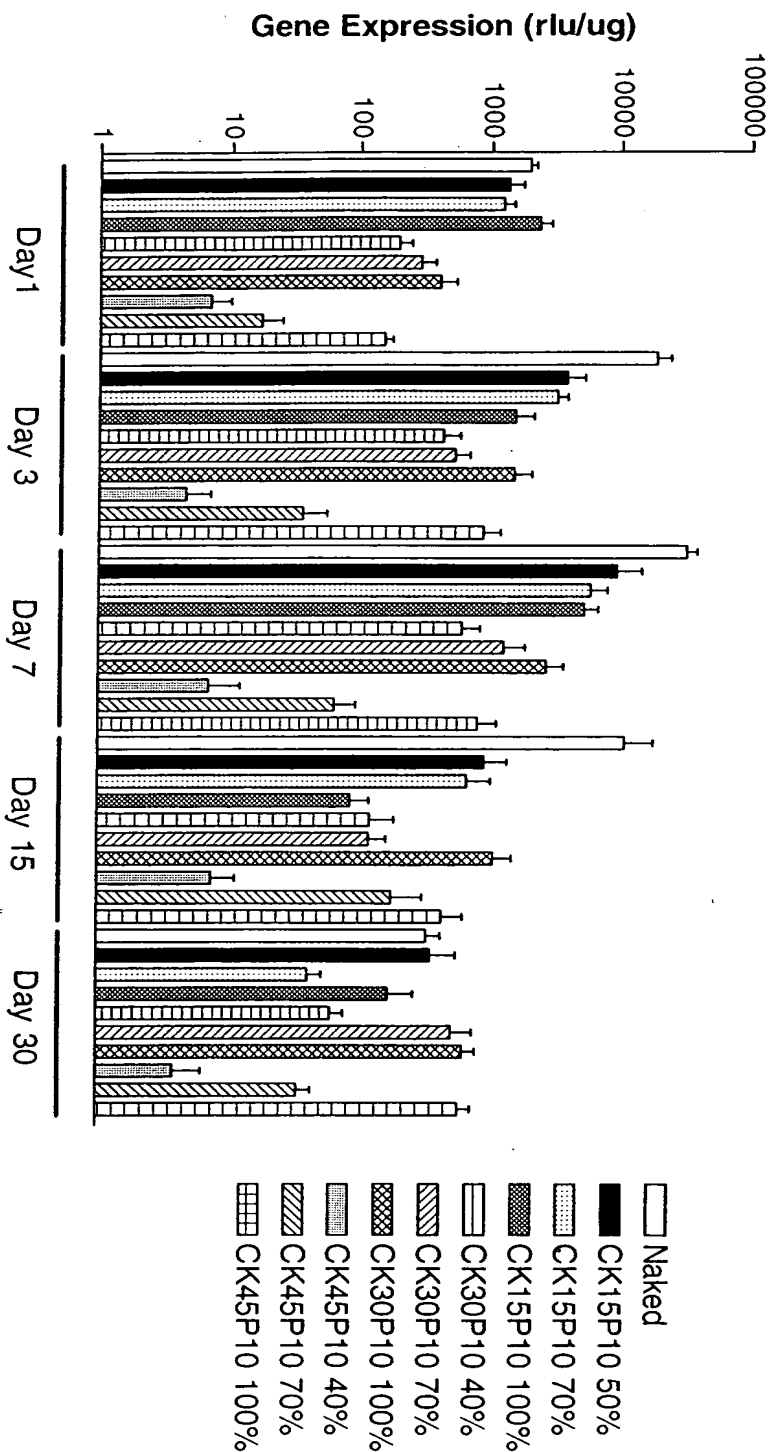
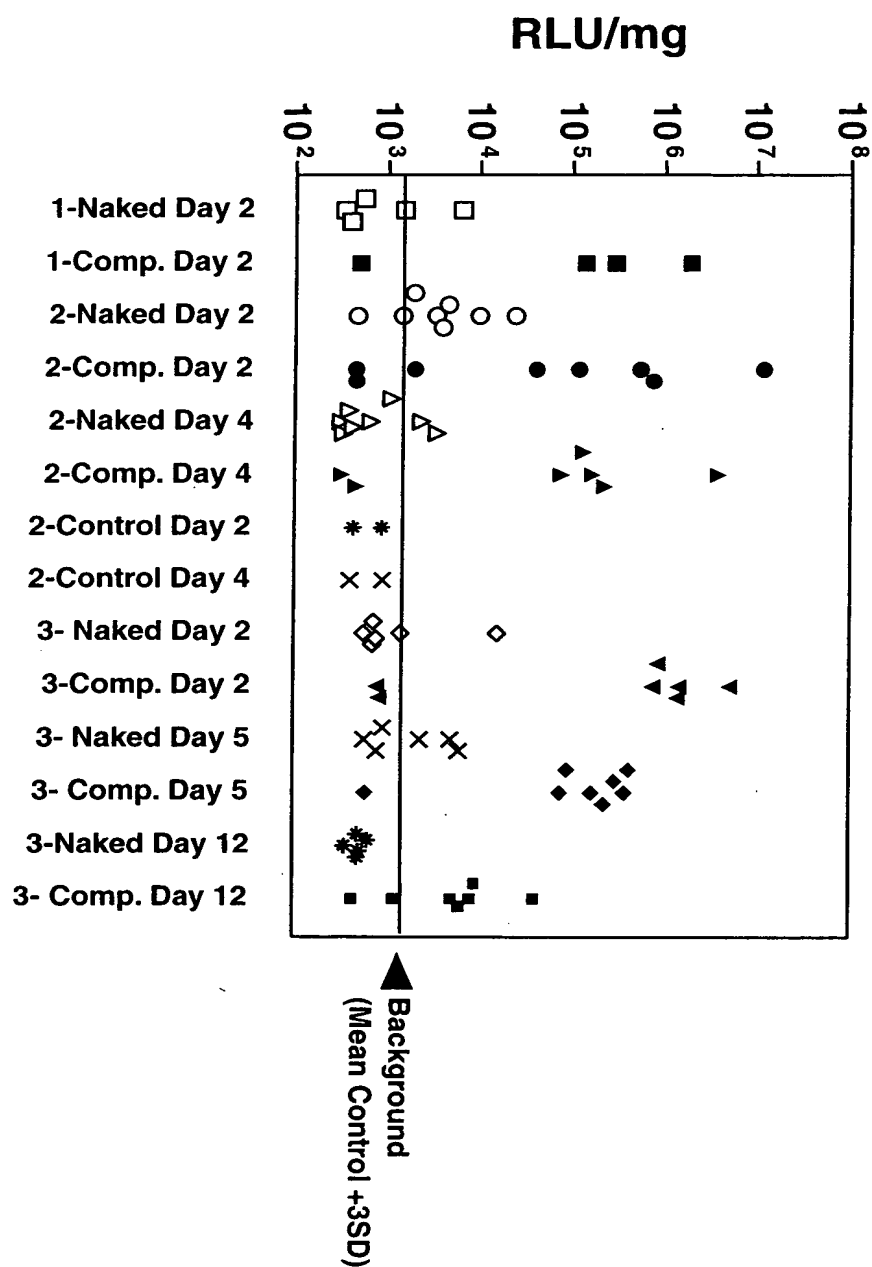


Fig. 5

Fig. 6



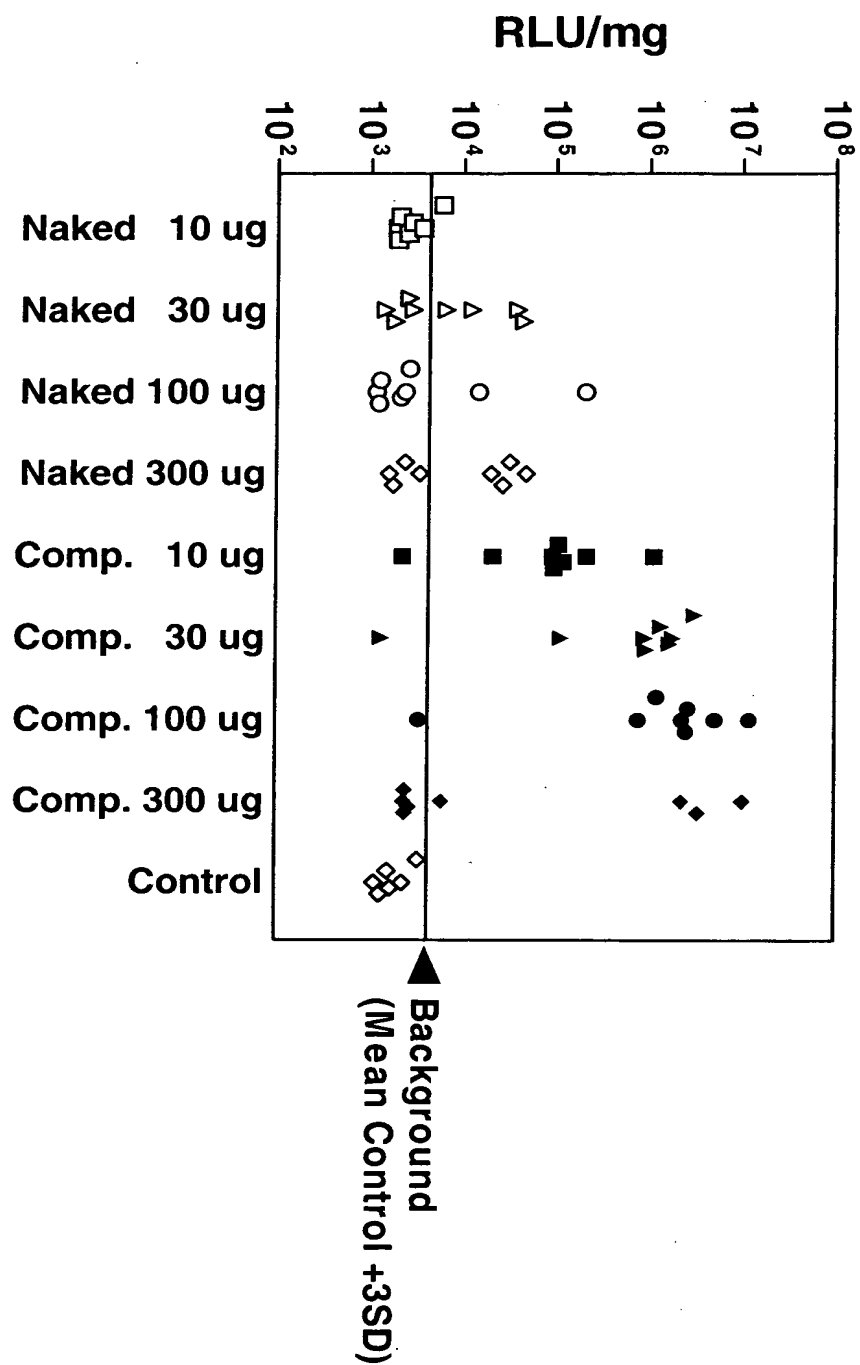


Fig. 7A

101630 6036360

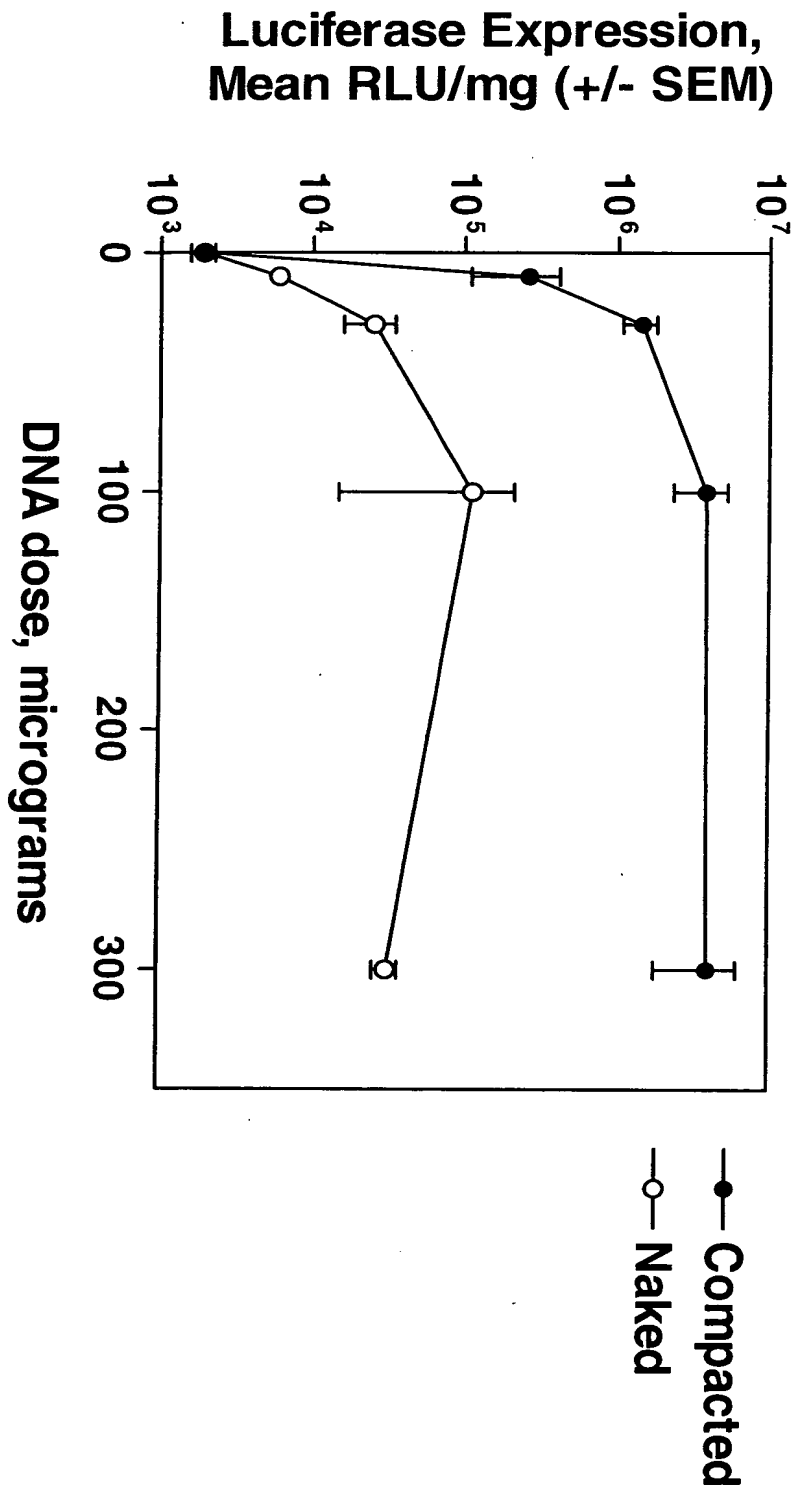


Fig. 7B



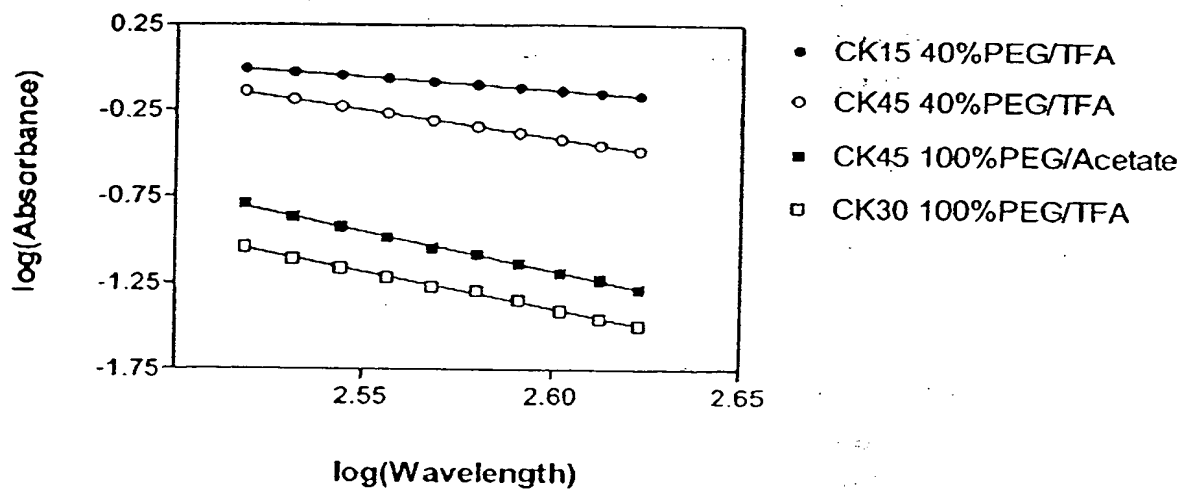
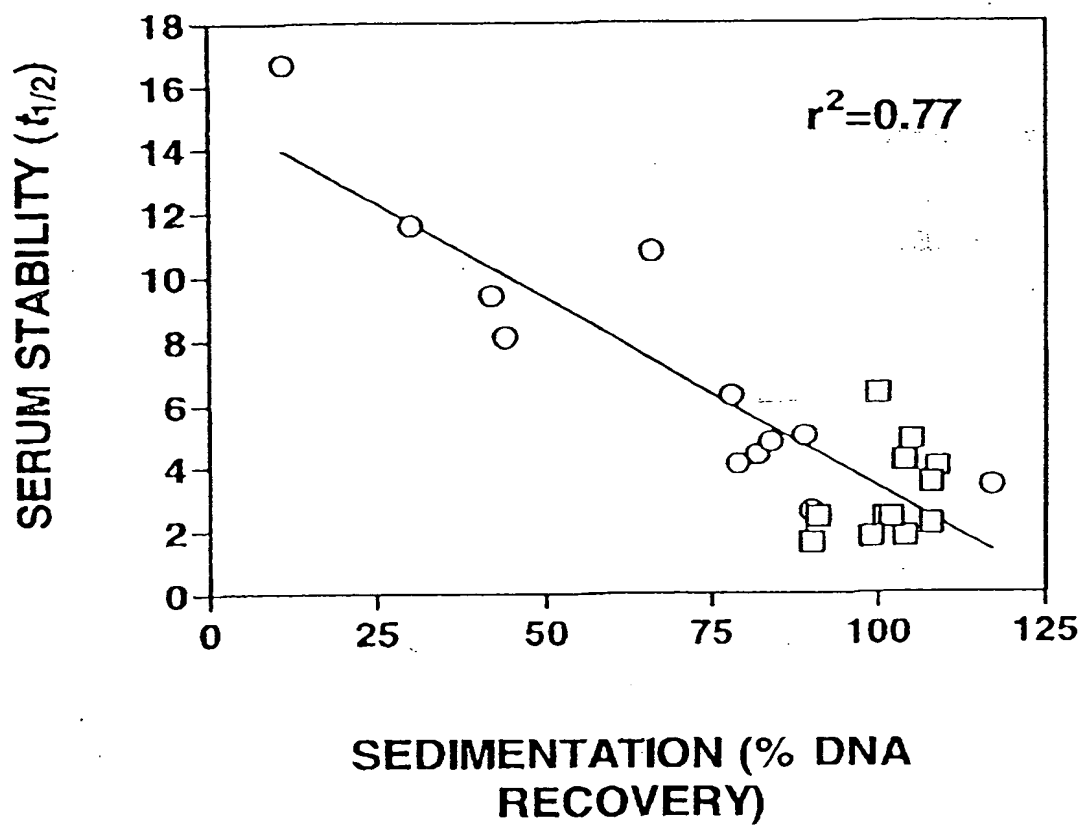
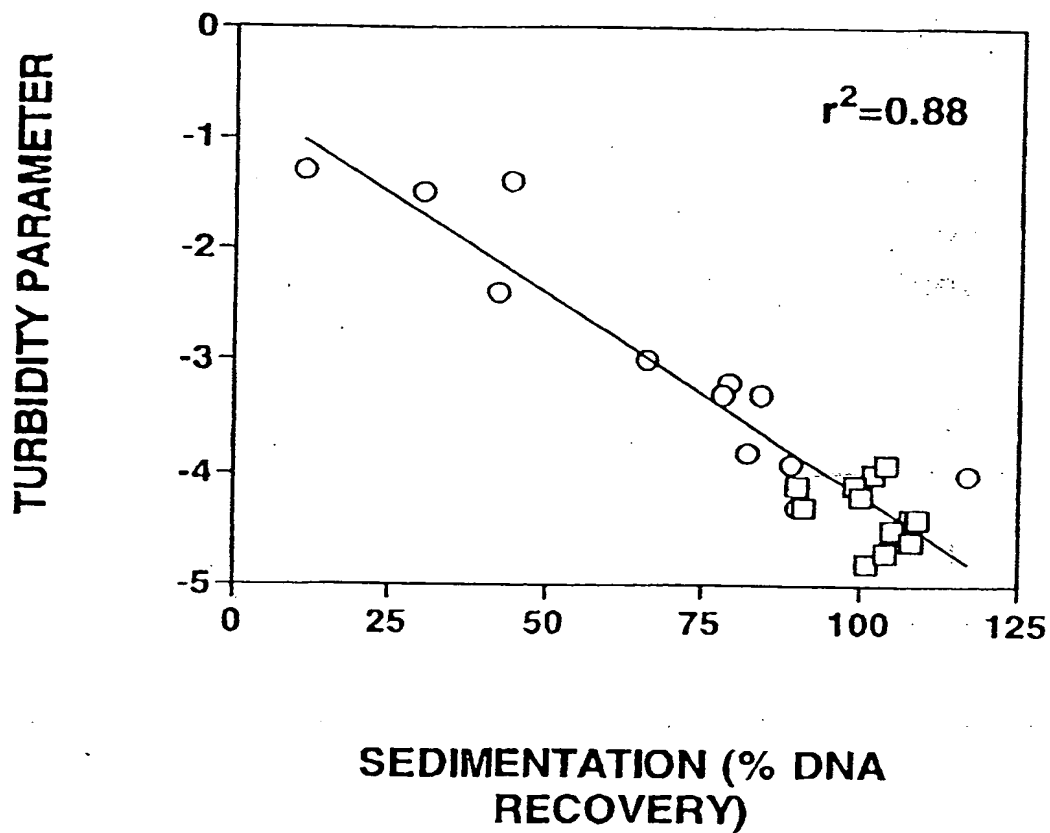


Fig. 8



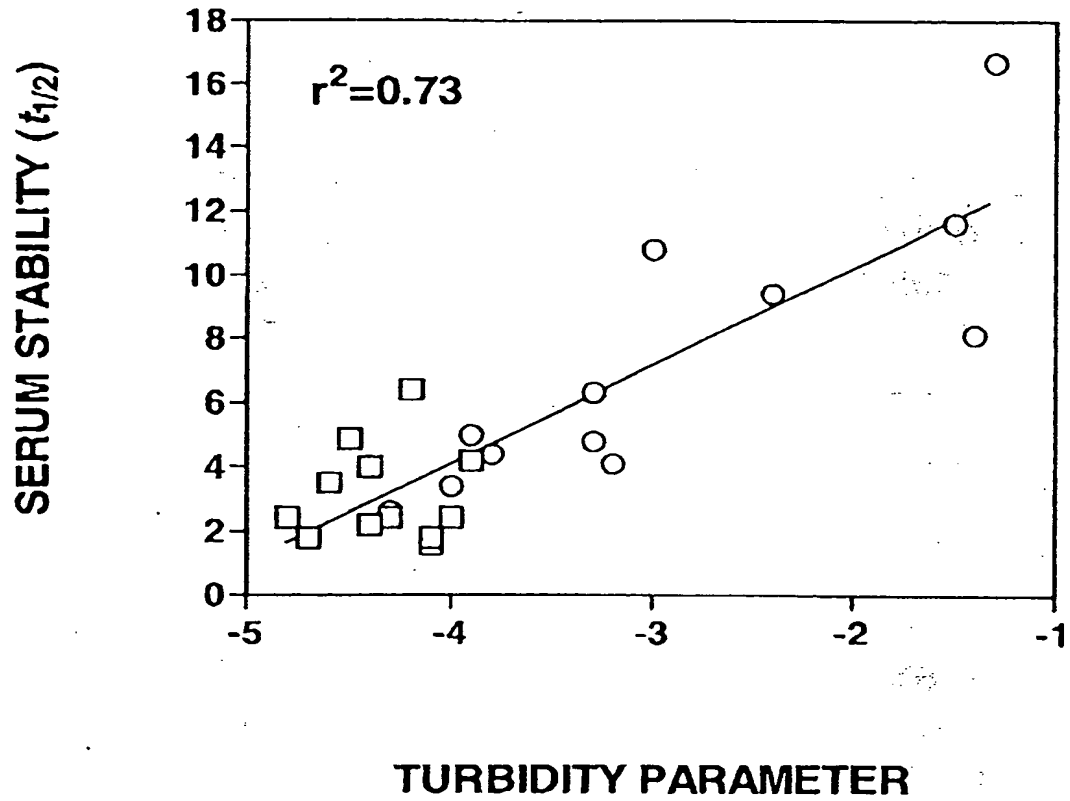
- Type A Formulations
- Type B Formulations

Fig. 9A



- Type A Formulations
- Type B Formulations

Fig. 9B



- Type A Formulations
- Type B Formulations

Fig. 9C

## PROPERTIES OF VARIOUS PLASmin™ FORMULATIONS

Formulation #	Counterion	Polylysine	PEG Content (%)	$t_{1/2}$ In Serum (h)	Turbidity Parameter	Sedimentation (%)
1	TFA	CK <sub>15</sub>	40	11.6	-1.5	30
2			60	10.8	-3.0	66
3			80	9.4	-2.4	42
4			100	16.7	-1.3	11
5			TFA	CK <sub>30</sub>	40	8.1
6	60	4.1			-3.2	79
7	80	3.4			-4.0	117
8	100	2.6			-4.3	90
9	TFA	CK <sub>45</sub>			40	6.3
10			60	4.4	-3.8	82
11			80	4.8	-3.3	84
12			100	5.0	-3.9	89
13			Acetate	CK <sub>15</sub>	40	2.4
14	60	1.8			-4.7	104
15	80	1.6			-4.1	90
16	100	2.4			-4.0	102
17	Acetate	CK <sub>30</sub>			40	1.8
18			60	2.4	-4.3	91
19			80	2.2	-4.4	108
20			100	4.0	-4.4	109
21			Acetate	CK <sub>45</sub>	40	6.4
22	60	4.2			-3.9	104
23	80	4.9			-4.5	105
24	100	3.5			-4.6	108

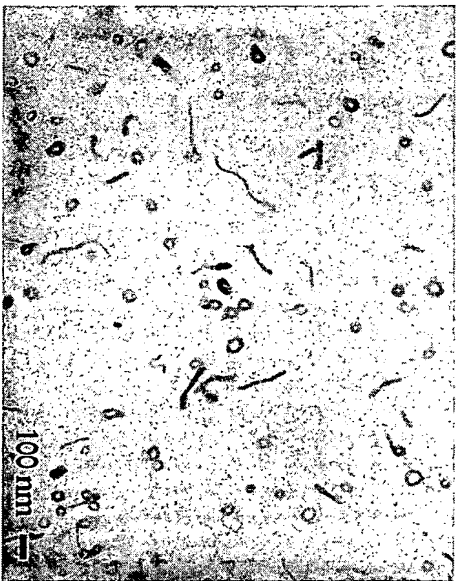
Fig. 9D

1016590 66925860

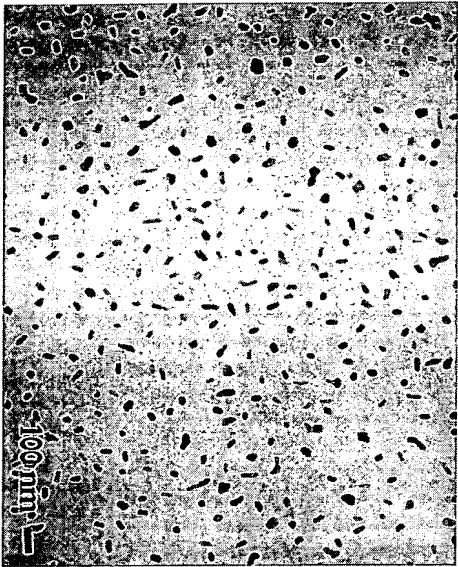
Acetate



HCO<sub>3</sub>



TFA



Chloride



Fig. 10

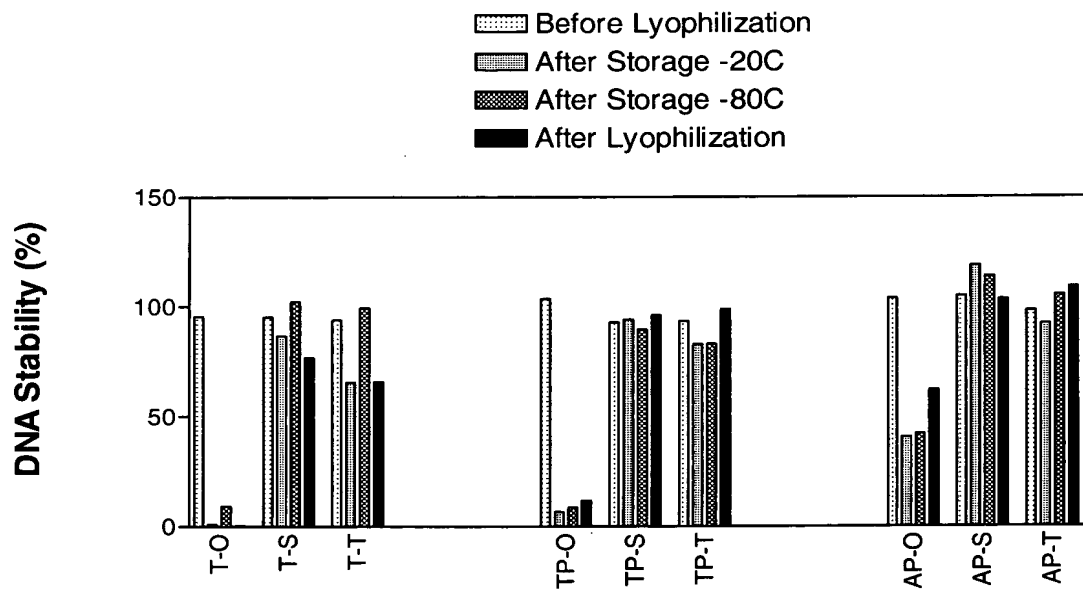


Fig. 11

Sample	Before Lyophilization	After Lyophilization
CK30TFA		
Original		
0.5M Sucrose	-4.31	ppt
0.5 M Trehalose	-3.81	-4.10
CK30P10k - TFA	-4.70	-4.01
Original		
0.5M Sucrose	-4.51	NE-4.61
0.5 M Trehalose	-4.15	
CK30P10k - Acetate	-4.65	-4.66
Original		-3.86
0.5M Sucrose	-4.76	
0.5 M Trehalose	-4.56	-3.32
	-4.57	-4.39

Fig. 12

09867593 0540  
107650 66929860



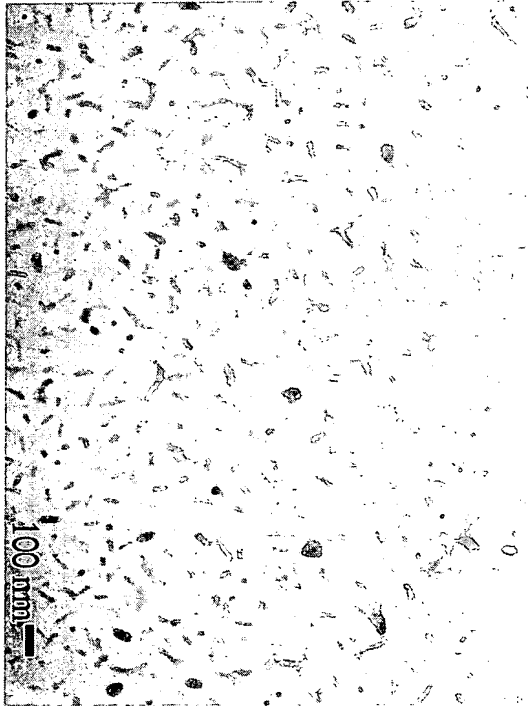
# AFTER



Fig. 13

107E50" 66925660

**BEFORE**



**AFTER**



Fig. 14

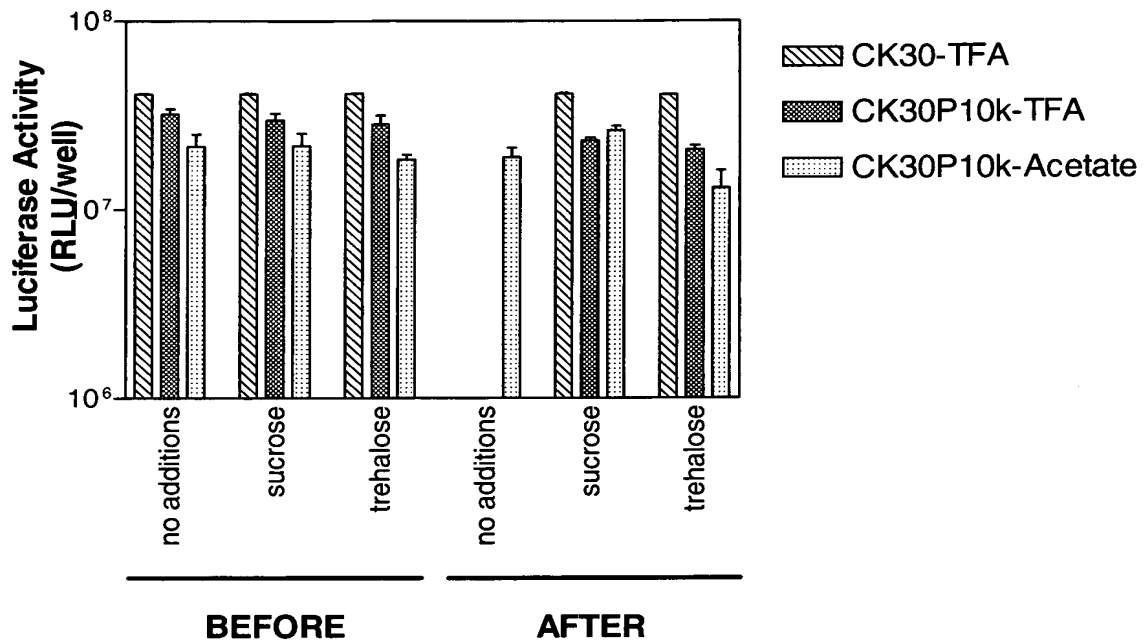


Fig. 15

Polylysine	Counterion	DNA Recovery	Turbidity Parameter
CK30P10k	Acetate	100	-4.2
	Bicarbonate	98	-4.0
	Chloride	101	-5.2*
	TFA	97	-4.6
CK45P10k	Chloride	105	-4.0

\* This value is lower than expected due to very low light scattering by this DNA formulation indicating that plasmid is not compacted, in agreement with electron microscopy and gel electrophoresis data.

Fig. 16

Magnification 40,000. The bar shows 100 nm

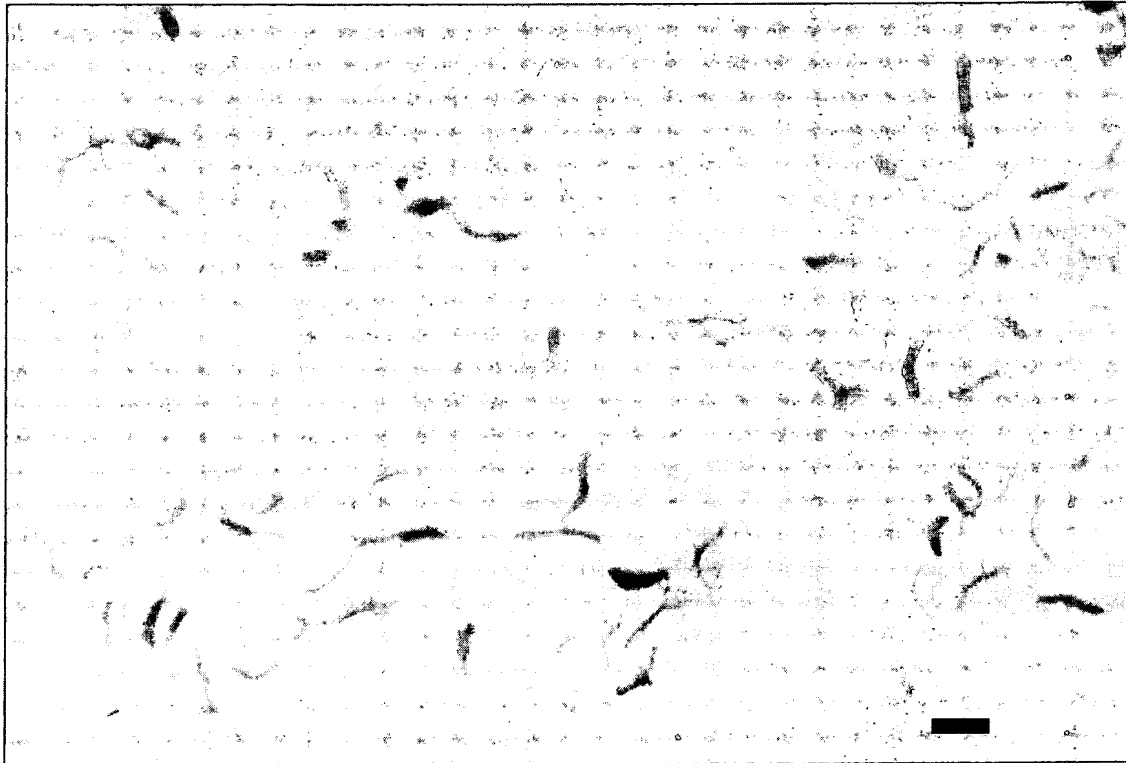


Fig. 17

Lane 1: DNA size markers.  
 Lane 2: naked DNA before compaction.  
 Lanes 3, 6, 9, and 12: compacted DNA.  
 Lanes 4, 7, 10, and 13: compacted DNA that was incubated in 75% mouse serum at 37 °C for 2 hr and trypsinized before loading.  
 Lanes 5, 8, 11, and 14: compacted DNA that was only trypsinized before loading.

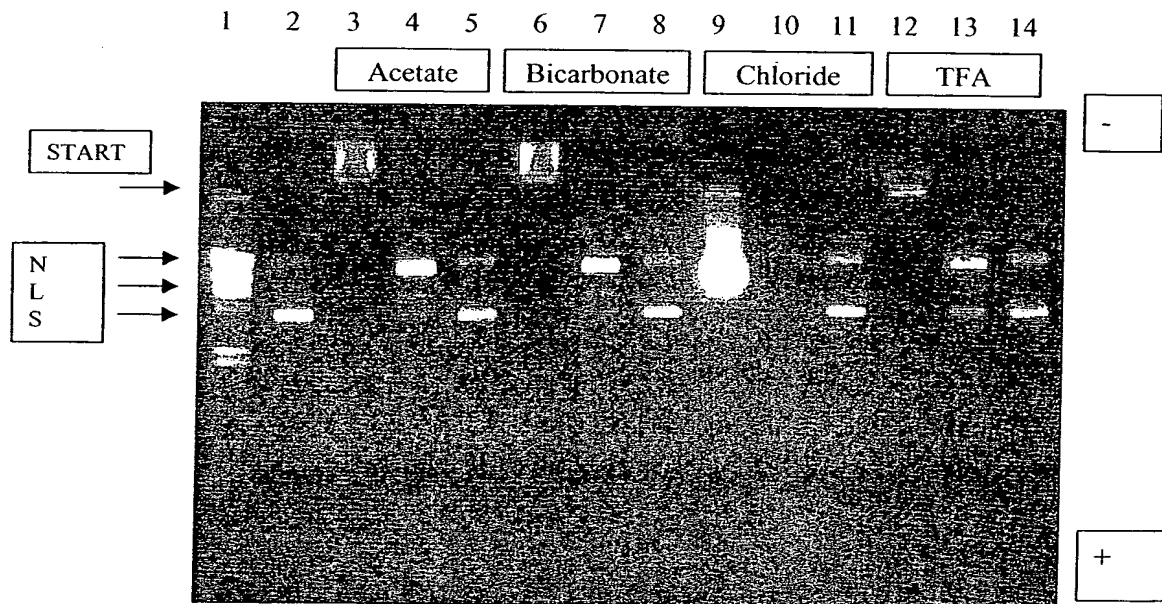
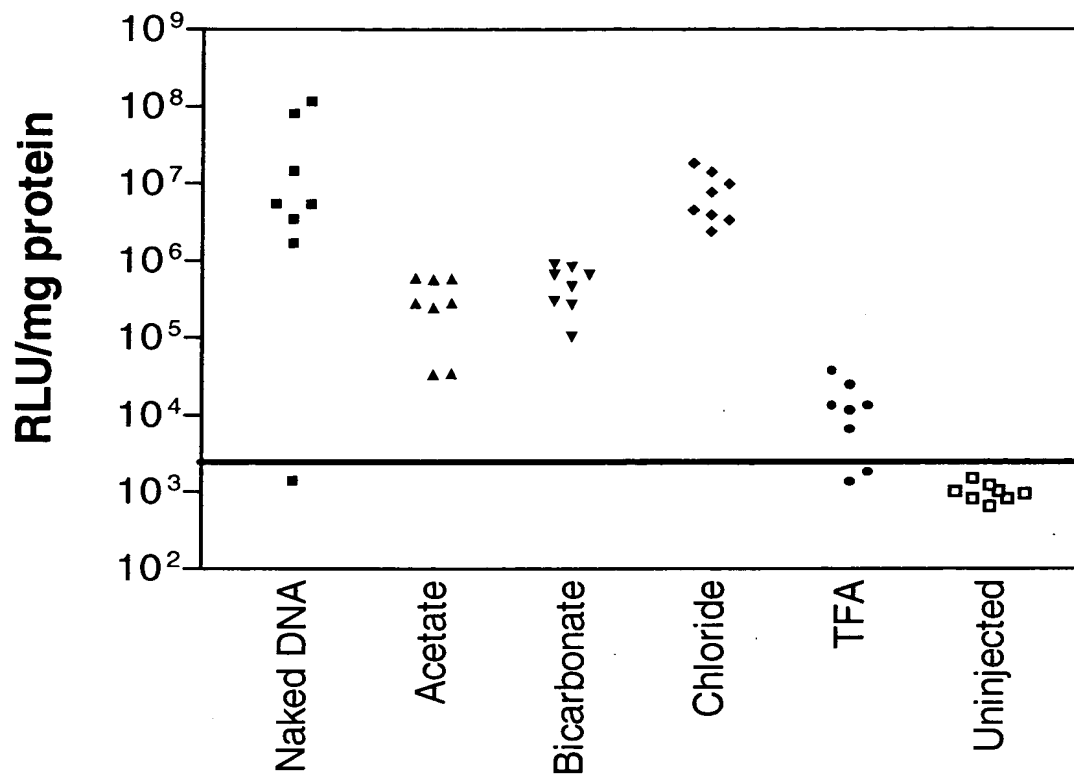


Fig. 18



Each point represents one animal. The solid line indicates background signal of luciferase assay. Dose 100  $\mu$ g DNA.

Fig. 19

Fig. 20